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Short communication

Evaluation of virucidal activity of fabrics using feline coronavirus

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) is an enveloped RNA virus responsible for the 2019 coronavirus disease (COVID-19) that represents a global health threat, causing an ongoing pandemic in many countries and territories. WHO recommendations emphasize the importance of all personal protective equipment (PPE) that can interrupt COVID-19 transmission. The textile industry and scientists are developing hygienic fabrics by the addition of or treatment with various antimicrobial and antiviral compounds. Methods for determining the antiviral activity of fabrics are reported in the International Standards Organization (ISO) 18184 (2019) guidelines.

Three different fabric samples treated with silver derivate, copper derivative and a not treated cotton fabric used as control were examined and put in contact with a suspension of feline coronavirus (FCoV). After 2 h of incubation a significant decrease of viral titer, as high as 3.25 log10 Tissue Culture Infectious Dose $(TCID)_{50}$/50 µl, in feline cells was observed in treated fabrics, with respect to not treated fabrics.

In this study, we optimized laboratory methods to evaluate the virucidal activity of silver- and copper treated cotton- based fabrics against coronavirus, using FCoV suitable as a surrogate of SARS-CoV-2 but safe for laboratory technicians.

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) is an enveloped RNA virus responsible for the 2019 coronavirus disease (COVID-19) that represents a global health threat, causing an ongoing pandemic in many countries and territories (Rodriguez-Morales et al., 2020). COVID-19 emerged in China and spread rapidly to other countries. Due to the severity of the infection and the potential of spreading on a global scale, the World Health Organisation (WHO), declared a global health emergency and the pandemic status. The virus affects the respiratory tract, with a number of patients displaying severe pneumonia and requiring hospitalisation and admission to intermediate or intensive care units.

The lack of effective antiviral drugs and vaccines (Pillaiyar et al., 2020) has underscored the need for containment measures, rapid diagnostic systems, quarantine and specific therapeutic treatments.

The principal routes of transmission of SARS-CoV-2 are respiratory droplets and direct contact person-to-person. Droplet transmission takes place when there is close contact (within 1 m) with a patient with respiratory symptoms and when potentially infective respiratory droplets reach the upper airway or the conjunctiva. This route may be possible in specific circumstances and mainly in healthcare settings in which procedures or support treatments generate aerosols (i.e. endotracheal intubation, bronchoscopy). Moreover, droplet particles can remain in the air for long periods and can reach other persons over distances greater than 1 m (Morawska and Cao, 2020). Transmission may also occur through fomites in the immediate environment around the infected person.

WHO recommendations emphasize the importance of all personal protective equipment (PPE) (i.e. medical masks, gloves and gown) that can interrupt COVID-19 transmission. Correct and rigorous behaviour of health care workers, hand hygiene and environmental cleaning and disinfection has been implemented especially in public places, hospitals and nursing homes. Therefore, maintaining physical distances and

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus type 2; COVID-19, 2019 coronavirus disease; WHO, World Health Organisation; PPE, personal protective equipment; ISO, International Standards Organization; FCoV, Feline coronavirus; TCID, Tissue Culture Infectious Dose; SD, standard deviation; CRFK, Crandell-Rees Feline Kidney Cell; D-MEM, Dulbecco's Modified Eagle Medium; cpe, cytopathic effect; ANOVA, one-way Analysis of Variance.

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avoiding strict, unprotected contact with persons with fever or respiratory symptoms is recommended.

SARS-CoV-2 and other human coronaviruses can survive for up to 9 days on contaminated environmental and inanimate surfaces. Contaminated surfaces are a potential source of viral transmission (Kampf et al., 2020), chiefly in healthcare settings, where highest viral load can be expected. Reducing viral load on inanimate surfaces is therefore a critical factor to contrast viral transmission.

The textile industry and scientists are developing hygienic fabrics by the addition of or treatment with various antimicrobial and antiviral compounds (Iyigundogdu et al., 2017). This has prompted the research to product fabrics and materials with intrinsic virucidal activity, with a wide range of efficacy and able to contrast the potential resistance of virus. Fabrics treated with virucidal substances have been implemented for PPE or to cover surfaces frequently touched or in contact with people. Recently, copper-based coating with polyurethane has been proven to reduce the persistence of SARS-CoV-2 on solids (Behzadinasab et al., 2020). Methods for determining the antiviral activity of fabrics are reported in the International Standards Organization (ISO) 18,184 (2019) guidelines in which influenza virus or feline calicivirus are used as virus models for enveloped and non-enveloped viruses, respectively. However, marked differences can be observed in terms of resistance to chemical and physical agents between different virus families and testing antiviral properties of fabrics against coronaviruses could be more appropriate. Feline coronavirus (FCoV) is a safe virus model for investigating in vitro coronavirus properties, including chemical-physical resistance and therapeutic strategies (Centers for Disease Control and Prevention (CDC, 2009). Some drugs currently introduced for treatment of SARS-CoV-2 have also been tested successfully in vivo in cats for treatment of FCoV-infected cats with severe clinical signs of peritonitis (Pedersen et al., 2018, 2019). In this study, we optimized laboratory methods to evaluate the virucidal activity of silver- and copper treated cotton- based fabrics against coronavirus, using feline coronavirus (FCoV) as surrogate of SARS-CoV-2.

2. Materials and methods

2.1. Virus

FCoV type II strain 25/92, isolated from a cat with infectious peritonis, was used for the virucidal activity tests. The virus stock with a titer of $10^{4.50}$ Tissue Culture Infectious Dose $(TCID)_{50}$/50 µl was stored at −80 °C and used for the experiments.

2.2. Cells

Crandell-Rees Feline Kidney Cell (CRFK), were cultured at 37 °C in a 5% CO_2 atmosphere in Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 10 % foetal bovine serum, 100 IU / ml penicillin, 0,1 mg/mL streptomycin and 2 mM L-glutamine.

2.3. Fabric samples

Three different fabric samples (A, B and C) were examined. Sample C was used as control for all the experiments. For the tests, samples aliquots of 50 mg each were subjected to sterilization with steam at 121 °C for 15′ prior to use.

2.4. Evaluation of cytotoxicity and non-specific virucidal activity of substances in fabrics

Fabric samples (50 mg) were immersed in D-MEM (10 mL) and shaken for 2 h at room temperature. Washing liquids were collected from each fabric (A, B, C) and used for the test.

Cytotoxicity of washing liquids was assessed on confluent monolayers of CRFK cells in 96-wells microtiter plates. After exposing cells to different dilutions (1:5, 1:10, 1:20, 1:40, 1:80) of the solutions A, B and C for 72 h of incubation the cytotoxicity of solutions was evaluated using inverted microscopy. Each experiment included untreated cells containing D-MEM (negative control).

Evaluation of non-specific virucidal activity of fabrics was carried out mixing washing liquids released from each fabric (A, B, C) in a 1:1 ratio with a FCoV suspension with a titer of $10^{4.50}$ $TCID_{50}$/50 µl. After 1 h contact at room temperature, each mixture was titrated. Non-specific virucidal activity of washing liquids was evaluated by measuring the difference in viral titer between solution from sample C set as control and those from samples A and B. The experiments were performed in triplicates.

2.5. Evaluation of virucidal activity of fabrics

Fabric samples (50 mg) were immersed in 10 mL of FCoV suspension with a titer of $10^{4.50}$ $TCID_{50}$/50 mL at room temperature. After 2 h of incubation, aliquots were collected and titrated.

Fabric virucidal activity was evaluated by measuring the difference of viral titer between sample C setted as control and samples A and B. The experiments were performed in triplicates.

2.6. Viral titration

Ten-fold dilutions (up to 10^{-6}) of each supernatant were titrated in quadruplicates in 96-well plates containing CRFK cells by endpoint dilution method.

The plates were incubated for 72 h at 37 °C in 5% CO_2 and the viral titers were determined on the basis of observation of cytopathic effect (cpe).

2.7. Data analysis

The values of virucidal activity assays were expressed as mean ± standard deviation (SD). Shapiro-Wilk test was used to assess the normality of distribution. Data were analysed by one-way Analysis of Variance (ANOVA) using Tukey test as *post hoc* test (statistical significance set at 0.05). Statistical analyses were performed with the software GraphPad Prism v 8.0.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Cytotoxicity and aspecific virucidal activity of substances in fabrics

The cytotoxicity of washing liquids from fabric samples was determined by microscopic examination of cell morphology after exposing the cells to various dilutions (1:5, 1:10, 1:20, 1:40, 1:80) for 72 h. The cellular morphological changes (loss of cell monolayer, granulation, cytoplasmic vacuolization, stretching and narrowing of cell extensions and darkening of the cell borders) resulted dilution-related.

The washing liquids from samples A (cotton fabric treated with silver derivative) and B (cotton fabric treated with copper derivative) showed cytotoxicity up to 1:40 dilution whereas fabric C (not treated cotton fabric) showed cytotoxicity up to 1:10 dilution. Comparing viral titer of the washing liquid from sample C (4.50 log10 $TCID_{50}$/50 µL) (control) and those from samples A and B a slight average decrease of 0.25 log10 $TCID_{50}$/50 µL was consistently observed, although this was not statistically significant.

3.2. Virucidal activity of fabrics

By comparing the viral titer of sample C (mean ± SD = 4.33 ± 0.14 log10 $TCID_{50}$/50 µL) with samples A (mean ± SD = 1.25 ± 0.25 log10 $TCID_{50}$/50 µL) and B (mean ± SD = 1.75 ± 0.5 log10 $TCID_{50}$/50 µL) a significant average decrease of the viral titer of 3.08 ($p < 0.0001$) and 2.58 log10 ($p = 0.0002$), respectively, was observed (Fig. 1). The

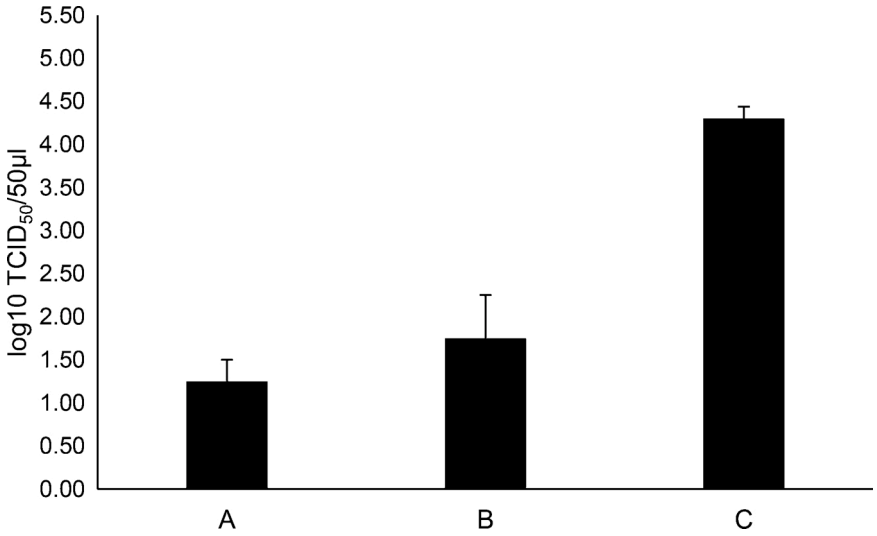

Fig. 1. Titers of Feline Coronavirus evaluated in Crandell feline cells and expressed as log10 $TCID_{50}$/50 µL. A: Viral titer after contact with silver-treated cotton-based fabric; B: Viral titer after contact with copper-treated cotton-based fabric; C: Viral titer after contact with not-treated cotton-based fabric (control).

ANOVA model showed a statistically significant effect of treatment of fabrics with copper and silver in the comparison based on the viral titration ($F = 73.94$, <0.0001).

4. Discussion

The global spread of SARS-CoV-2 pandemic has imposed the adoption of prevention measures based mainly on physical distancing and on the use of individual protective devices. In order to avoid or decrease the possibility of indirect contagion through contaminated surfaces, the use of disinfectants or other products with virucidal activity is rapidly being adopted.

For preventing transmission of pathogens in hospitals, antimicrobial fabrics with self-disinfecting function have been implemented (Meng et al., 2016). Different antimicrobial finishing methods have been developed (Budama et al., 2013) in the textile industry based on the use of novel fabrics, particularly in hospital environment where blood, body fluids, and secretions, easily in contact with cotton fabrics, are possible reservoirs of pathogens. Accordingly, the demand for fabrics with verified virucidal activity is quickly increasing not only in healthcare system but also in public areas (i.e. restaurants, schools, theatre, public transportation, etc).

The aim of this study was to set up technical protocols useful to assess the virucidal activity of different treated fabrics against coronavirus. We assessed the virucidal activity of silver- and copper treated cotton- based fabrics against FCoV, since this coronavirus is expected to be more reliable as virus model for SARS-CoV-2 than influenza virus and feline calicivirus in terms of physico-chemical characteristics.

An important aspect of treated fabrics is the preliminary evaluation of the possible release of substances, which could lead to irritation and allergy issues when in contact with the skin (Jantas, 2006).

In our study, the cytotoxicity of washing liquids from fabric samples was determined after exposing CRFK cells to various dilutions. The washing liquids from samples A and B showed cytotoxicity up to 1:40 dilution whereas for fabric C cytotoxicity was observed up to 1:10 dilution.

Shaking of fabrics in D-MEM also ruled out the presence in the fabric of soluble substances with virucidal activity that could misrepresent the actual virucidal activity of the fabric.

A limit of our study was that applied strictly the ISO protocol without assessing variables such the effects of time contact and virus titer, which could provide more hints into the antiviral activities of fabrics against coronaviruses. The virucidal activity of fabrics against FCoV was evaluated by measuring the difference in viral titers between sample C (control, not treated) and samples A and B. We observed a significant decrease of viral titer of samples A and B with respect to sample C, ranging between 3.25 and 2.75 log10 $TCID_{50}$/50 µl, respectively. Based on International standards ISO18814:2019, virucidal activity of sample A should be considered excellent whilst the virucidal activity of sample B should be considered as good.

5. Conclusions

In this study we demonstrated that the protocol is suitable to unveil the virucidal activity of treated fabrics against coronaviruses. Moreover, silver and copper-treated fabrics were able to reduce significantly the titer of FCoV. This virus could be used in laboratories as a safe surrogate for SARS CoV-2 (Yang et al., 2020).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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Authors' contributions

MC and CB drafted the manuscript and assisted with the conception and design of the study, data collection, and data analysis. GL assisted with study design and protocol development and assisted in writing and editing the manuscript. VM delineated the hypothesis, helped conceive and design the study, performed and oversaw the data analyses, and assisted in the writing of the manuscript. MSL, ND and CC assisted with data analysis and interpretation of the study.

All authors read and approved the final manuscript for submission and publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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